Cellular Mechanisms in Differentiation and Growth

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V. INFECTION AND HEREDITY

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THE present territorial dispute between infection and heredity might have been predicted from the bridging of the gap between microbiology and genetics. Having once learned in bacteriology that "germ" is infectious microorganism, the student finds in genetics that "germ" is hereditary constitution and the confusion persists. Our factual discussion will rely upon some recent examples from the genetics of enteric bacteria, but many authors could testify against any pretense of innovation in the theme (Darlington, 1944; Medawar, 1947; Sonneborn, 1950; Ephrussi, 1953).[2]

I. GENETIC ANALYSIS IN BACTERIA

A. Recombination mechanisms. Enteric bacteria have joined the list of model organisms for genetic research. Much of our story will concern a particular strain, K-12, of *Escherichia coli,* which was the first to be used for cross-breeding analysis in bacteria (Tatum and Lederberg, 1947; Lederberg and Tatum, 1953).

Microscopy is still an unreliable method for the detection of mating processes in *E. coli,* and we look instead for genetic signs, for cells that display new combinations of genetic traits where two parents that differ in a number of characters are grown together. It is often convenient to use genetic markers that are easily selected for or against, such as drug resistance or requirement for growth factors, for they allow recombinants to be selected at will from populations in which they are greatly outnumbered by unmated parental cells. However, highly fertile stocks are now available with which selected markers are no longer required to demonstrate recombination.

Genetic recombination can be achieved by other processes besides sexual fertilization, notably *transduction,* which is the transmission of a small fragment of genetic material from one cell to another. The physical

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[2] The literature references in this review are not necessarily primary documentation and are chosen as most economically leading to the detailed sources.

and genetic criteria by which these mechanisms can be distinguished are summarized as follows:

Recombination Mechanisms

Mechanism	Genetic Element	Material Agency
Sex	Many linked markers (whole genome or nucleus)	Fertilization by an intact cell (gamete)
Transduction	Single marker or cluster of closely linked markers (chromosome fragment)	Transfer of subcellular (filtrable) material (DNA or virus particles)

By both criteria, recombination in *E. coli* K-12 is sexual (Lederberg, 1955). As our knowledge of these mechanisms increases, it may become advisable to refine our classification. For example, one can conceive of partial fertilizations that might be achieved by subhaploid or partially inactivated gametes (Muller and Pontecorvo, 1940; Briggs, 1952). On the other hand, Pontecorvo (1954) has proposed the term "parasexual" for any mechanism of recombination that does not partake of typical *secondary* sexual paraphernalia. The present classification adheres to nuclear fusion as the essential element of sexuality (Link, 1929), which is indeed the root of its genetic significance.

More recent microscopic studies have related recombination in K-12 to the formation of conjoined pairs of bacterial cells. The conjugants do not copulate in their entirety but eventually disjoin (Lederberg, 1956b); the relationship of conjugation to mating type is elaborated in the next section. Direct cytological evidence of karyogamy still remains to be obtained. The current controversy over the cytological definition of bacterial chromosomes will not be reviewed here; they will be postulated as the material counterparts of genetic linkage.

B. Sexual reproduction and the mating type system. The first attempts to cross K-12 (Tatum and Lederberg, 1947) were made with some trepidation. So many microorganisms have evolved mating type systems (recently reviewed by Raper, 1954; *Neurospora crassa* was the immediate example) that we feared we might have to test many strains in all combinations, a task beyond the technical resources of the time. But strain K-12 proved to be self-compatible, and the first experiment worked very

well. Many other strains have been studied since, and, in retrospect, the chances of that success were only about 0.02. Although such a figure is usually translated "statistically impossible," it is only a minor hindrance to Dr. Tatum's well-known serendipity.

That strain K-12 was self-compatible was indicated (Lederberg et al., 1951) by several facts—not only were all the original stocks derived from this strain mutually compatible, but no segregation of compatibility preferences was found among their first and second generation progeny. In hopes of finding a mating type system elsewhere, about 2,000 strains of *E. coli* that had been isolated from various sources, from turkey feces to human pus, were screened for sexual fertility by a simplified method. About 40 fertile strains had been found and were about to be tested further when a compatibility pattern emerged in K-12 itself. At the same time, we encountered an apparently sterile "mutant" and Dr. L. L. Cavalli at Milan, with whom we had been corresponding, discovered that one of the original stocks was actually self-sterile. Our collaborative studies (Cavalli, Lederberg, and Lederberg, 1953; Lederberg, Cavalli, and Lederberg, 1952) have supported the following interpretation:

The wild type strain of K-12 is self-compatible and is designated F^+. Most of the derivative stocks are likewise F^+. However, rare "mutations" for compatibility have led to the self-sterile type, F^-. Of the possible combinations, $F^- \times F^-$ is sterile, while $F^+ \times F^+$ and $F^+ \times F^-$ are progressively more fertile. A pair of standard F^+ and F^- strains can thus be used to type an unknown culture by means of the test crosses with it.

As stated before, segregation of compatibility, of F^+/F^-, had not been observed in previous crosses, although many of them had been $F^+ \times F^-$. Further experiments confirmed that $F^+ \times F^-$ crosses gave only F^+ progeny, a behavior unlike that of any other trait that has been studied in *E. coli*. The answer to this puzzle came from experiments to look for a possible hormonal stimulus from F^+ cells that might allow two otherwise incompatible F^- cultures to mate with one another. This was tested by mixing genetically labelled cultures so that different matings would give different kinds of offspring. In such mixtures, it was found that the F^- strains had been impelled to mate with each other, but this proved to be much more than a simple physiological stimulus, as the restoration of compatibility was permanent, genetically irreversible. That is to say, if an F^- strain is simply grown in contact with F^+ cells, the F^- becomes permanently F^+. The conversion is extremely efficient and occurs almost

as frequently as the F^+ and F^- cells can be calculated to collide with one another. It is therefore not surprising that the progeny of $F^+ \times F^-$ crosses become uniformly converted as if by venereal infection

The F^+ quality is extremely contagious—it will eventually spread through an entire F^- population seeded by an F^+ cell—so that it would be plausible to suppose that there is an F^+ agent or virus responsible for its spread. But repeated experiments have failed to detect any infectious particle other than the intact F^+ cell, despite the sensitivity of the test for a single particle. For example, the two mating types have been grown on opposite sides of a thin-rolled membrane filter, about 15μ thick and with pores about 1μ diameter, barely capable of holding back the bacteria. Although Dr. Grobstein, who kindly furnished the filters, has demonstrated embryonic inductions through them (1953), no passage of the F quality could be demonstrated. The F conversion in addition to its genetic quality thus appears to correspond to the contact transformations which have repeatedly been encountered in developmental studies (Weiss, 1947; Spratt, 1954; Cantino and Horenstein, 1954; Sussman and Sussman, 1956). In common with these examples, it is not known whether there is a material exchange of matrix, surface, or cytoplasm, and the expression *F^+ agent* or *particle* must be remembered as being a figure of speech.[3]

A number of mating type variations have been discovered in K-12, but cross-infection experiments have shown these to depend on the genetic constitution of the bacterium rather than on varieties of the F agent (Lederberg, unpublished; Cavalli and Ceppellini, 1953). For example, F^+ stocks vary in their apparent potency and can be gradated so that the most fertile combinations are those most widely separated. However, the conversion of a given F^- stock by contact with any one of these F^+ types results in F^+ derivatives of the same potency. Nevertheless, some evidence for variability in F among different strains, independently isolated from nature, has emerged from experiments in which K-12 F^- testers

[3] M. Delbrück, in discussing this question at the symposium, has brought to mind reactions of very high molecular order; for example, the rate of activation of phage T_4 is proportional to the 5th power of the concentration of tryptophane (Stent and Wollman, 1950). Such reactions if they occur between a cell producing the stimulus and another receiving it will display an equally abrupt dependence on the distance between the cells, and can thus account for contact transformations. However, an organized particle or patch of cell surface can be thought of as a mechanism of coordinating the elements of a high order reaction so that it will occur at a significant rate, the probability of random coincidence of the units being otherwise small. The kinetic description is not, therefore, a contradictory alternative to surface interaction theories, but it should further attempts to accomplish such transformations by high concentrations of cellular fractions, or synthetic analogues.

had been converted to F^+ by the foreign strains. It has been repeatedly noticed that such F^+ stocks lose their compatibility when stored for a few weeks on nutrient agar slants, in contrast to the stability of intra-strain conversions.

TABLE I. Mating types in *E. coli.*

Item	Type	Compatibility with standard F^- (W-1177)	Can be "infected" [2] with F^+ from K-12	Can infect standard F^-
1	K-12 F^-	—	+	—
2	K-12 F^+	+	o	+
3	K-12 F^+ aeration phenocopy	—	o	+
4	K-12 Hfr[4]	+++	—	—
5	K-12 F^+ weakly compatible	±	o[1]	+
6	K-12 and others F^- refractory	—	—	—
7	foreign strains F^+	+	o	+ (unstable)
8	foreign strain [3] sterile F^+	—	o[1]	+
9	foreign strain compatible F^-	+	+	—
10	foreign strain compatible, refractory F^-	+	—	—
11	most strains of *E. coli* [3] and other bacteria intersterile F^-	—	—	—

[1] The compatibility reactions of these strains is unaltered by exposure to an F^+ culture.

[2] The susceptibility to conversion is tested by passing the F^+ quality in turn to a standard F^- unless the strain is already F^+, as well as by change of sexual reactivity. The symbol O indicates no test.

[3] Unlike the others, these strains are infertile in crosses with F^+ as well as F^- stocks.

[4] (Cavalli, 1950).

It has been suggested (Hayes, 1953) that the F agent functions in *E. coli* crosses as an extracellular vector of the gametic genes, by analogy with the role of phage in genetic transduction in *Salmonella*. In view of the abject failure of all attempts to demonstrate an extracellular agent of F conversion or recombination, this proposal is no longer current in its original form, though it may be semantically equated to fertilization by identifying the F converting agent with the F^+ cell, which is operationally correct.

The relationships between compatibility and F status have, furthermore, proved to be surprisingly complicated for an organism that was once thought to be homothallic, as illustrated by the mating types listed in Table I. These relationships show that an infective F^+ agent is neither necessary nor sufficient: the reactivity of a strain is controlled alternatively by its own constitution and by its F status. The environment also plays a role, as is shown by the aeration phenocopy: F^+ cultures can be made to simulate the reactions of F^- by cultivating them under strong aeration. These cells, however, retain the F agent and the effect is completely reversible when clones are regrown under standard conditions. At first, no method was available for the intentional production of F^- strains, and the discovery of the system had to wait upon the sporadic occurrence of two unselected mutants. P. D. Skaar (unpublished) has discovered, however, that the passage of motile F^+ strains through soft agar often results in the development of F^- variants. The mechanism of this effect, even whether it is inductive or selective, is entirely unknown, but it may be related to the continued rapid division of well dispersed bacteria at the growth frontier in this medium, by analogy with the loss of *kappa* from rapidly grown clones of *Paramecium* (Beale, 1954). At any rate, the F^- stocks obtained by this technique have been indispensable for further analyses (Nelson and Lederberg, 1954).

Hfr cultures have also been extremely useful for further studies, owing to their high frequency of recombination, and the non-contagious control of compatibility. It was first thought that Hfr $\times$ F^- crosses gave exclusively F^- progeny, but it has since been found (Cavalli and Jinks, 1956) that the two alternatives segregate, with a low frequency of Hfr, and this marker being linked to another locus called Gal, of which more is to be said.

These crosses are also the basis of recent observations of conjugation. That crosses in *E. coli* might be physiologically polarized had already been suggested by Hayes' (1953) observations on the effect of streptomycin, which, in the course of inactivating bacteria for vegetative growth,

also sterilizes F^- cells sexually. F^+ cells, however, retain some sexual reactivity. Although this was initially attributed to the extracellular persistence of postulated, extruded F^+ agents, it could equally well have been speculated that the F^- gamete contributes the bulk of the cytoplasm to the zygote while the F^+ gamete leaves behind the poisoned cytoplasm at conjugation.

That the differential effect of streptomycin is actually related to sexual differentiation is now indicated by microscopic study of Hfr $\times$ F^- conjugations. The Hfr exconjugants have given pure, unaltered clones, while the recombinants have issued exclusively from the F^- exconjugants, together with unaltered F^- cells. Fertilization thus appears to involve the passage of a nucleus from the Hfr cell to the F^- cell, wherein one fertilization nucleus or zygote is formed. The remaining nuclei of the multinucleate parental cells are unaltered, accounting for the persistence of both parental types. In sum, the process is not greatly different from hyphal fertilizations in molds, though no sexual spores have been observed. Previous studies had already demonstrated the haplobiontic life cycle with a haploid vegetative phase and a diploid zygote, which undergoes immediate reduction. If such concepts are transferable at all, it may be permissible to regard the F^- mating type as female, and the F^+ or Hfr as phenotypically determined hermaphrodites. Obligate male stocks have yet to be found, but could be detected only by attempting crosses between two males. At present, the likely point of difference between F^+ and F^- is at the bacterial surface and the ability to pair, but it may be anticipated that other steps of the sexual process are likewise subject to genetic control as in other fungi (Wheeler, 1954, and note item 8 of Table I).

The most perplexing feature of K-12 genetics is the polarized segregation so that sexual progeny tend to resemble the F^- parent more closely than the F^+ or Hfr parent. A wide variety of suggestions has been adduced to explain this fact, but they can be divided into two groups: (1) that the F^+ gamete is already defective, so that the contribution to the zygote is less from the F^+ than from the F^- side (Hayes, 1953; Wollman, 1953) and (2) that the F^+ gamete contains a full genetic complement, but that losses occur later, preferentially from the F^+ contribution to the zygote, (Nelson and Lederberg, 1954; Cavalli and Jinks, 1956; Lederberg, 1955; Cavalli, Lederberg, and Lederberg, 1953). Unfortunately, in most experiments, we can only put the parent cells together and observe the segregants that issue forth, and it is manifestly impossible to decide precisely when in the interval the evident losses have occurred.

For this reason, most of the experimental data that have been brought to bear on this problem are indecisive in so far as they deal with haploid segregants.

The fortuitous discovery (after calculated searches had failed) of unreduced, diploid sexual progeny, and of "Het" stocks that tend to generate diploids on further crossing, has led to an independent approach (Lederberg, 1949a; Nelson and Lederberg, 1954). Before the compatibility system was detected, the peculiarities of these diploids had already demonstrated that segments of chromosomes were being lost during the sexual cycle. These segments directly involve only a few markers, but a chromosome of which a piece is missing will be inviable when it stands alone in a haploid cell. Therefore, not only the deleted markers, but any loci that may be linked to them, will be affected in segregation. A detailed study of these diploids has shown (a) that the regions subject to deletion are quite invariable; (b) that the deletion always occurs; (c) that although it tends to occur on the F^+ parental chromosomes, it will sometimes occur *instead* on the F^-, from which it may be concluded (d) that the occurrence is postzygotic. To be sure, other aberrations may be postulated, too, but these are sufficient to account for the facts and to exhaust one's credulity. Furthermore, no diploids have ever been found that would correspond to the variable losses that have been proposed by other authors. Most recently, Wollman and Jacob (1955) have reported that mechanical disruption of a mating mixture at various times will influence the segregation pattern. They deduce that fertilization is normally fractional and progressive for different parts of the genome. It is not clear, however, whether the postulated fractional fertilization occurs normally or is artificially induced. Moreover, to explain the haploid segregation pattern, indirect effects on chromosome pairing and postzygotic losses could just as well be supposed. For a definite determination, it will be necessary to find unreduced partial diploids, whose occurrence and structure can be inferred only ambiguously from seeing only the final pool of segregants from many zygotes.

C. Lysogenicity and transduction. A second germinal element of *E. coli* K-12 is a bacteriophage, *lambda*. Like F, this element could be discovered only when a mutant cropped up that was an indicator for it. For twenty-five years, the bacteria had harbored this phage without revealing it to many students who had examined K-12 as a typical strain of *E. coli*. That bacteria might be lysogenic, that is maintain phage as an intracellular symbiont, had been suggested already by d'Herelle, and in the early 1930's, Burnet expressed a clear insight into the genetic im-

plications of the fact of lysogenicity. During the past five years, lysogenic systems have again attracted the interest of many virus workers (Lwoff, 1953; Luria, 1953). Burnet showed, and it was more rigorously confirmed later, that lysogenic bacteria do not contain intact virus particles; neither do sensitive bacteria soon after they have been infected with the virus. We therefore assume that the genetic element of the phage can develop in the bacterium in a latent form, the "prophage" in the lysogenic bacterium. When, under the influence of the total genotype of the infected cell, the somatic envelope is manufactured, and the virus nucleus enclosed in it, the latent phage matures into infectious particles, ready to reinitiate the parasitic cycle. In lysogenic bacteria, this transition occurs only sporadically, so that the over-all viability of the lysogenic strain is scarcely affected, but under the influence of ultraviolet light, nearly every prophage can be induced to mature, with concomitant lysis of the bacterium.

The first experimental findings on *lambda* (Lederberg, E., 1951) were (1) the parent K-12 strain and most of its descendants are lysogenic for *lambda;* (2) sensitive variants occasionally appeared among the survivors of ultraviolet irradiation (selected or induced?); (3) sensitive bacteria could be infected with *lambda,* whereupon most of the bacteria would lyse and release a new crop of the virus; however, perhaps a tenth of the bacteria gave viable clones from which lysogenic strains could be isolated.

The demonstration of lysogenicity in a sexually fertile strain opened the way to analysis of its genetic basis, that had formerly been the subject of considerable speculation. Probably most workers had an a priori conception that lysogenicity was a cytoplasmic infection (Lederberg, 1949b), analogous to the killer phenotype in *Paramecium* which depends on a factor called *kappa.* However, the results of crosses between lysogenic and sensitive strains soon showed (Lederberg and Lederberg, 1953) that lysogenicity depended on a chromosomal locus (or segment), Lp, prominently linked to another locus, Gal (for galactose fermentation).[4] The most striking bit of evidence was the synthesis of diploid heterozygotes which segregated both factors in linkage, with occasional recombinants. In addition, crosses of a *lambda*-lysogenic with another stock, carrying

[4] Some reservations with which this interpretation was first received (based on questions of F polarity, Wollman, 1953; Lwoff, 1953) appear to have been dispelled (Wollman and Jacob, 1955). They were founded not on any statistically significant discrepancies in experimental results, but upon the ambiguity of haploid segregation data already discussed. Fortunately, the behavior of heterozygous diploids answers or circumvents any questions that might pertain to the content of the original gametes.

a different phage, gave recombinants of all types, including the doubly sensitive combination. These results were incompatible with the simple cytoplasmic concept of *lambda* infection, but they do not rigorously prove that more than an indispensable component, not necessarily the entire prophage, is localized at the Lp site. Subsequent experiments by Appleyard (1953) and others have, however, shown that at least some genetic markers of the *lambda,* as well as lysogenicity itself, are localized near Gal. It is a plausible inference that the entire prophage consists of a chromosome segment of the lysogenic bacterium.

In our experience, the presence or absence of *lambda* has had no appreciable effect on segregation of Gal and other markers, unlike the decisive role of F polarity. Jacob and Wollman (1954) have, however, reported a curious interaction among their strains: when lysogenic Hfr was mated with "ly$^-$" (sensitive?) F$^-$, most of the zygotes lysed, with the production of free *lambda,* as if the prophage had been induced to mature in the course of fertilization. This effect, under their experimental conditions, may possibly be attributed to the combination of the prophage from the Hfr cell with the fresh sensitive cytoplasm of the F$^-$ conjugant, which would be comparable to the act of ordinary infection. Lysogenic $\times$ lysogenic crosses did not show the effect. The suggestion that this syngamic induction may equally well be the origin of anomalies such as segmental elimination therefore does not concord with previous studies by various workers, both on diploids and haploid segregants, which have almost uniformly involved only lysogenic parents, or with similar genetic results in sensitive $\times$ sensitive crosses.

At first, no genetic correlate of *lambda* in *E. coli* was observed. However, in the course of experiments designed to test for recombination in another enteric bacterium, *Salmonella,* a mechanism was found that was distinct from sexual fertilization. This proved to be an example of genetic transduction (defined in a previous paragraph as the transfer of a genetic fragment) in which bacteriophage particles conveyed genetic factors from the bacteria on which they were grown to new hosts (Zinder and Lederberg, 1952). Contemporaneously, the well-known pneumococcus transformation (Griffith, 1928) had begun to receive close attention in its genetic aspects and to be understood as, in effect, the historically first example of transduction, though here DNA functions directly without the benefit of a special vector. In *Salmonella,* the phage stands in for the biochemist in shattering the chromosomes of the host cell and introducing them into a recipient bacterium.

The role of phage in *Salmonella* transduction impelled a renewed study

of a possible similar role of *lambda,* in *E. coli,* notwithstanding the previous negative results. It was found (Morse, 1954, 1955; Morse, Lederberg and Lederberg, 1956) that *lambda* would transduce the Gal factor, but no others so far known. The two systems may be contrasted in several respects: (1) In *Salmonella,* nothing is known of the localization of prophage, and any genetic locus can be transduced; in K-12, the prophage is located at Lp, and only the Lp-linked factor, Gal, is transduced by *lambda;* (2) In *Salmonella,* phage is equally competent for transduction whether it is grown directly on sensitive hosts or is obtained from the ultraviolet-induced lysis of lysogenic strains; In *E. coli, lambda* is competent only when prepared by UV-induced lysis. These differences suggest that the relationship of the phage to the transduced segment is adventitious in *Salmonella,* but more direct in *E. coli.* A third difference is of another order: in *Salmonella,* transduction is promptly consummated and the transformed clone shows no residue of the segment that had been replaced; in *E. coli,* the fragment may persist indefinitely, and reproduce as such side by side with the homologous recipient chromosome.

These cells which carry an extra fragment, are called *heterogenotes.* The extra fragment itself is an *exogenote.* In heterogenotic clones, from time to time, "crossing-over" does take place between the exogenote and the intact chromosome. Whether this exchange involves physical breaks, or a modification of the replication process is no better known for transduction than for crossing-over in higher forms. The segregants may then resemble either the original recipient parent, or the strain on which the phage had been grown, or both. The persistent heterogenotes thus give insight into the intermediate stages of transduction, which proceed too rapidly for analysis in *Salmonella.*

The phage obtained from typical haploid lysogenic clones has a transduction competence of about 1 per 10^5 phage particles. By contrast, the phage obtained from heterogenotes has a competence of from 10 to 100%. That is, after correcting for virus that may have issued from segregant bacteria, virtually every phage particle from a heterogenote carries the genetic qualities of the exogenote with it. From this it can be speculated that the exogenote is the prophage itself: it is, after all, the direct descendant of a fragment that had been introduced by a previous phage infection, and which has been stringently selected for its ability to support the function of the Gal^+ gene. We may imagine that the low competence of the usual lysates reflects the unlikelihood that a random fragment will have been broken out with the right size and shape.

On this line of argument, infection and lysogenization may be considered a special case of transduction, the prophage having the dual aspect of virus nucleus and chromosome segment. Whether *lambda* originated by the mutation of a chromosome segment of an aboriginal *E. coli,* or is the reduced and integrated relic of genetic material of external origin, hybrid or parasitic, is a question in paleobacteriology that may never be answerable.

D. Gene action and position effect. In the previous discussion, Gal was referred to as a single locus controlling galactose fermentation. As seems likely to happen for any genetic locus that is studied closely enough, recurrent galactose-negative mutations have proved to be non-identical (pseudoallelic) and a series of closely linked loci can be recognized by recombination test, although the mutant phenotypes are virtually indistinguishable (E. Lederberg, 1952). In most instances, heterogenotes compounded from two distinct mutants have shown the normal galactose-positive phenotype. In some combinations, however, these heterogenotes are galactose-negative, although, having a structure $+-/-+$ (Gal_x^+ Gal_y^-/Gal_x^- Gal_y^+) they bear altogether a full complement of Gal^+ genes. Heterogenotes of the structure $++/--$ can also be synthesized, and these are galactose-positive. These combinations thus display an unmistakable *cis-trans* position effect (Lewis, 1955), that is, the two Gal^+ genes can effectively interact only in a *cis-* and not in a *trans-*arrangement, as between exogenote and chromosome (Morse, 1955).

As there are at least ten distinct loci in the Gal cluster, and probably many more, it will be interesting to look for some pattern in the position relationships, and this is currently in progress. A second type of position effect might be thought of, a possible functional distinction between genes in the chromosome as compared to the exogenote. This would amount to position effects between the loci in the exogenotic region, and loci in adjacent regions, but so far has not been detected. Position effects are the most direct manifestations of primary gene interactions and are often hypothetically explained by the interplay of nondiffusible gene products. Transduction analysis has unexpectedly led to a new approach to this fundamental problem of genetics.

Demerec (1955) has applied similar techniques to the analysis of pseudoallelic relationships in *Salmonella,* having found that mutations whose effects are biochemically related are closely linked. The startling inference that the linear sequence of these mutations corresponds to the biosynthetic sequence of reactions has also been forwarded, but the detailed numerical data in support of this inference have not yet been pub-

lished. Similar correlations between the position and function of various genes have been sought, without success, in several organisms (Cf. Sturtevant and Schultz, 1931). The examples of pseudoalleles in *E. coli* (E. Lederberg, 1952; Morse, 1955) and in fungi (Pontecorvo, 1952; Mitchell, 1955) must be distinguished from Demerec's series, as the same biochemical defect, so far is known, is associated with all the pseudoalleles in these cases. The physiological significance of such correlated sequences is even more obscure if they are unique for some processes in *Salmonella.*

When the biochemical genetics of *Neurospora* was first being developed, ca. 1940-1945, the evidence from nutritional mutants was considered to favor an elementary correspondence between single genes and single enzymes. However, semantic and experimental ambiguities have emerged (J. Lederberg, 1951; Wagner and Mitchell, 1955) and many students have now adopted a more agnostic attitude to this doctrine. Therefore, serial correlations between biochemical lesions and mutant positions (even if more than fortuitous) cannot safely be translated into an assembly line of enzyme syntheses, and might well have more to do with the functional integration of the unit steps than with the specificity of the catalysts. Whatever final interpretation is placed on these studies, they illustrate the potency for phenogenetic exploration inherent in the tools for recombination analysis in bacteria.

II. MODELS OF DEVELOPMENT

The quasi-irreversibility of differentiated clones of cells in development has posed a riddle as provoking to geneticists as it is to embryologists (Weiss, 1947). Many genetic studies with microorganisms have been motivated by their application as models of development. The genetic importance of the cytoplasm has been roundly confirmed by such studies on *Paramecium* and on yeast (Sonneborn, 1950, 1954; Ephrussi, 1953). But the corresponding de-emphasis of the developmental role of the nucleus is less warranted. These ancillary studies may help to suggest some of the possible theories that should be considered for development. Which are correct must be learned by asking the questions of embryos rather than microbes.

However *Salmonella* has furnished a probable model example of quasi-irreversible genic changes. It has been known since Andrewes' work (1922) that the flagella of this genus occur in two antigenic phases. The antigenic phase is almost stable in clonal multiplication, but an occasional cell in one phase suddenly initiates a clone of the alternative phase. The rate of transition is highly variable from one strain to an-

other, from an almost negligible frequency to as high as once per thousand cell divisions (Stocker, 1949). Altogether, some hundred different flagellar antigens are known, and occur in various combinations in different strains, but the phases of a given strain are constrained to a single pair of alternatives. This restriction already distinguishes phase variation from ordinary mutation, since we observe no mutation in antigenic specificity, only a choice of which of two alternatives will be expressed.

Transduction analysis of the flagellar antigens (Lederberg and Edwards, 1953) has confirmed that two independent loci, H_1 and H_2, control the antigenic potentialities of each strain; at each of these loci there are many alternative alleles, the combination of one H_1 and one H_2 allele defining the serological type of any strain. Thus, *Salmonella typhimurium* has the immunogenetic constitution H_1^{1} H_2^{2}, while *Salmonella abony* is H_1^{b} H_2^{enx}. Any given clone of *S. typhimurium* will, however, contain cells of either the 1- or the 2- antigenic type. What is the genetic basis of the difference between these clones of differing phase? This question can be answered in part by transduction experiments on the H_1 and H_2 loci, involving various combinations of phases of *typhimurium* and *abony*. If the phase were controlled by a cytoplasmic state, or by chromosomal factors not linked to H_1 or H_2, then the outcome of these experiments might depend on the phase of the recipient, but would be independent of the phase of the donor. In fact, the outcome does depend on the phase of both the donor and the recipient. We therefore infer that whatever element controls the phase is coupled, during transduction, with either the H_1 or the H_2 locus, or both. The details of these experiments are rather complex, and have not been fully completed or analyzed, but the H_2 locus appears to be decisive. That is, which of the two antigenic possibilities is realized seems to depend on a quasi-irreversible differentiation of the H_2 locus. The simplest speculation to rationalize the alternation of states at H_2 is that the local accumulation of the immediate products keeps this gene active, to produce more of the same, and suppresses or competes with the H_1 gene.[5]

The analysis of this genic differentiation is too flimsy, by itself, to stand as an effective model of differentiation. McClintock's (1951) work on maize has exposed an elaborate system of local genic modification, and King and Briggs (1955) have recently carried their studies of nuclear transplantation to the indisputable conclusion that the nuclei of

[5] A more detailed study which shows that phase variation depends on alternative states of the H_2 locus has now been completed (Lederberg and Iino, 1956).

developing embryonic cells in the frog are genetically altered. By further elaboration of their techniques and those of microbial genetics, it may ultimately be feasible to analyse the genetic differences among differentiated tissues no less exactly than is possible for the clones of mutant microorganisms (Lederberg, 1956a).

III. GENETIC PARTICLES

The main prop of formal genetic explanation is the "self-reproducing particle." In the last century, the problem of growth and reproduction was transposed from the whole organism to the cell, then regarded as the ultimate unit of biological structure and function. Intracellular constituents, most notoriously the genes, are now assigned the same role of the fundamental self-reproductive element of which the growth of the whole organism is the summation. But what is a "particle" and what does "*self*-reproduction" mean?

The structure of an elementary particle is a paradox the physicists have ever had to contend with. Likewise, as their instruments achieve higher resolution, biologists have had to reconcile themselves to new orders of complexity even in such atomistic units as genes. In genetics, "particle" is used in two senses: an abstract inference from breeding data and a microscopic object. Unfortunately, the correspondence of the formal and material units has rarely, if ever, been proven.

A. Formal particles. The geneticist usually infers a particle from discontinuities in inheritance: segregation in sexual progeny, mutations, unequal cell divisions. Thus Mendel was able to deduce the basic laws of diploid inheritance from the results of crosses with peas without considering the material nature of his Anlagen. Mutational discontinuities, independent for different qualities, led many students to adopt a particulate theory of inheritance in bacteria before this could be confirmed by recombination technique (Luria, 1947; Lederberg, 1948). In yeast (Ephrussi, 1953; Spiegelman, 1951) particles have been inferred to explain discontinuities in the transmission of a trait in vegetative reproduction, after the example of Sonneborn and Preer with *Paramecium* (Beale, 1954). The evaluation of target number and size in radiobiological experiments has been a fashionable exercise, whether or not the implied targets had any independent standing as real biological units, leading sometimes to absurd conclusions (Lea, 1947). The outstanding success of Mendelian analysis has possibly blunted the general criticism that these particles are only formal descriptions of cell division, a mathematical simplification that leaves untouched the question of their materiality.

This criticism is most realistic for particles that have not been directly visualized or for which inconsistencies have emerged. For example, Ephrussi's study (1953) of the induction of respiratory-deficient mutants, among the buds of yeast cells exposed to acriflavine, has implied that little of the cytoplasm of the mother cell is passed to the bud. From a technically analogous study of another trait, adaptation to galactose, Spiegelman (1951) inferred an equipartition of the maternal cytoplasm.

Unequal cell divisions generally (including stem cells, indeed the primary act of differentiation) have been and are apt subjects of a formal particle analysis, but primarily to suggest material hypotheses that can be independently checked. For a microbial example, in experiments on the transduction of motility to nonmotile strains of *Salmonella* (Stocker, Zinder, and Lederberg, 1953; Lederberg and Stocker, 1955), cells that had acquired the motile phenotype could be isolated and followed directly under the microscope. Only a small fraction of these isolates generated simple motile clones, corresponding to the previous knowledge of transduction of other markers. Most of the initial motile cells gave clones in which motility was transmitted to a limited number of cells, from 1 to 100. These motile cells in turn generated unbranched chains of descent. At each successive fission of the motile cell in such a hereditary chain, one motile and one nonmotile daughter were produced, a segregation so sharp that it was at first thought to be certain evidence of the persistence of a nonreproducing particle. How 1 to 100 such particles would be generated in one clone will be discussed elsewhere. We may still question here whether the bald description of unequal division is not more informative than the postulation of a motility-conferring particle. The so-called particle might simply be the mathematical representation of a rule of cell division, that the locomotive machinery is not randomly divided, or if it divides at all, the lesser parts are incompetent. The particle hypothesis does lead to certain lines of inquiry (e.g. whether chain cells are uniflagellate), but we must also remember how little we know of the mechanics of cell division as it pertains to cytoplasmic structure in any organism.

Unequal division is a regular feature of the vegetative growth of certain diatoms: the cell wall consists of two rigid half-walls, one fitting inside the other like the halves of a Petri dish. At cell division, the half-walls separate, and a new half-wall is secreted *within* each. The previous inner half, which is the smaller, thus serves as the outer half-wall at the next generation, and this cell is therefore smaller than its sister. The average cell size of a clone thus becomes progressively smaller, but may

be restored through the sexual auxospore stage (Wiedling, 1948). The morphological description of cell division saves us from a rather elaborate particle formulation which might otherwise be invoked to account for the size classes and progressive diminution in a diatom culture. Alternatively, we are reminded that the formal "particle" does not only imply a material granule, but a polarized process at cell division.

Numerical data on the partition of cellular organelles that might bolster the usual hypothesis of random partition are unfortunately very scanty. Wilson (1931) has recorded some figures on the partition of the 24 chondriospheres in the spermatocyte to the spermatids, in a scorpion. The partition is inexact, but although Wilson quoted it as random, the distribution is actually much more compact.

Another interpretation of particles is in the chemical terms of a steady state, alternative chains of reactions being assumed to compete with one another (as suggested, for example, by Delbrück; see Beale, 1954). This mode of formal description can apply, among others, to real particles too, the mathematical laws of competition being relevant even to populations of free living organisms. This formulation is therefore not, as has been erroneously suggested, an alternative hypothesis to particles, but a more general, and possibly more fruitful mode of description (Pollock, 1953). Efforts to find experimental discriminations are therefore likely to be foredoomed by tautology.

B. Visible particles. (For documentation of the following section, see J. Lederberg, 1952.) At least since Altmann's bioblasts, various particles that have been seen within cells have been imbued with genetic functions. Unfortunately, the imputation has rarely been backed by critical proof. Three kinds of inclusion may be considered: the chromosomes, mitochondria and other organelles, and endosymbionts. The last two are distinguished in principle by the postulated identity of the latter with independent organisms, but as this is a matter of techniques and definition, all extra-chromosomal particles that function in heredity may be classified together as plasmids.

That the chromosomes are the material sites of the formal genes is no longer disputed. There is no question of the exact correspondence between the two constructs throughout the life cycle of many organisms and under the most exhaustive experimental stresses. To anticipate the following discussion of plasmids, can we, however, postulate a third invisible element of which the chromosomes and the formal genes in their linear groups are both subordinate manifestations? In a sense, we do if we postulate, as many authors have, that only a part of the chromosome

has genetic functions, the remainder being inessential to its basic continuity. If there is a third element, it would have to be the invariable companion of the chromosomes everywhere, e.g. in the compact sperm head; the most dedicated critics of the chromosome theory have exhausted themselves in efforts to separate the ideal from the real chromosomes.

The genetic quality of the plasmids is much more doubtful. The most convincing correspondences apply to those plasmids that can be cultivated outside their usual hosts, such as the rickettsial symbionts of arthropods or the yeast-like symbionts of beetles, though these have generally been remembered for their pathogenic or nutritional rather than genetic functions. For other plasmids, formal particles have been inferred on other evidence, but the correspondence rests on very shaky evidence, if any. The problem is not very different from that of the etiology of infectious disease, which in the early development of bacteriology, lay in chaos before Robert Koch had presented his famous four postulates: (1) The microbe must invariably accompany the disease. (2) It must be isolated from the diseased tissue and grown in pure culture. (3) The pure culture must reproduce the disease when reinoculated in healthy animals. (4) The same microbe must be reisolated from the artificial infection. If plasmid is substituted for microbe, and phenotype for disease, the applicability of these postulates in genetics is obvious. For present day technology, the second criterion may be too stringent, and chemical purification may be substituted for pure culture. In that event, the validity of the proof will rest on the reliability of the purification, and on indirect evidence that the particle can grow *in vivo*.

Kappa in *Paramecium* perhaps best illustrates the use of these criteria (Beale, 1954). The number and size of (formal) *kappa* particles was estimated before they fed the hope that *kappa* could be visualized. By microscopic observation, particles were then discovered which satisfied all but the second criterion, as cross-infection was accomplished only by conjugation, not from pure culture. Logically (if not very plausibly), it can still be argued that the true genetic element is undiscovered, and that *kappa* (like paramecin in turn) is an epiphenomenon. More recently, it has been possible to transmit *kappa* efficiently by cell-free homogenates, which if sufficiently purified may serve for the second criterion and complete the proof (Sonneborn, personal communication).

Other plasmids, especially the chloroplasts, have been identified by less secure inferences. In many plants, breeding tests have shown that the presence or quality of chloroplasts may be maternally, presumably cyto-

plasmically, controlled. The chloroplasts are the most prominent inclusion in the cytoplasm, but there is no other evidence that they are the genetic element in question. An analogous statement applies to the respiratory granules in yeast (Ephrussi, 1953). Since chloroplasts can be removed from many plants by treatment with streptomycin, there is an attractive opportunity to try reinfection experiments which have not hitherto been reported. The mitochondria are still less certain, as cells that have been deprived of their mitochondria are not likely to be viable. However, Lettré has stated that devitalized ascites tumor cells that had been cytolysed in distilled water could be resuscitated by artificial reinfection with suspensions of granules. Unfortunately, the experiments could not be regularly reproduced, and it is too soon to ask further questions, whether the granules are "self-reproductive elements" or repair the damaged cells only in a physiological sense. Where viability is the sole phenotypic effect, such a distinction may be difficult, but one can study the specificity as to source of the reparative material. Another approach is suggested by LeClerc's experiments (1954) on enzymatic stimulation in chick chorioallantois treated with liver microsomes. Her data do not, however, show whether the granules have multiplied.

C. Self-reproduction. Living and self-reproducing (SR) are probably synonymous concepts, though one is often used to explain the other; the semantics (meaninglessness) of "life" and "living" has been ably exposed by Pirie (1937). The prefix "self" is the stumbling block to useful understanding, since to detect reproduction (without self) is often only a problem in arithmetic. SR must be interpreted as some degree of self-sufficiency, but with respect to what? If SR is to have any material meaning, it must apply at least to whole organisms, and these we know are dependent on the outside world at least for the energy and substance of reproduction.

SR might, as the next resort, mean self-sufficiency in information or specificity, but again the least exacting autotrophic organism must obtain its substance in specific chemical forms, and has a negligible probability of survival if randomly situated in the universe. A great deal of information is inherent in the specifications of the terrestrial biosphere. We are accustomed to discounting these nutritional problems, and might consider an entity as (almost) SR if it proliferates on a medium whose chemical composition is (almost) definable. Alternatively, self-sufficiency is, at best, a relative concept, the inverse of the least specificity required of the environment. To date, chromosomes and most plasmids cannot be cultivated *in vitro,* and we know nothing of their demands on the host

cytoplasm, whether they are of such a different order from the nutrition (information input) of more familiar microorganisms to warrant a unique classification on other than technical grounds.

If their self-sufficiency is only relative, chromosomes (and plasmids) are assuredly self-necessary or self-dependent, that is to say the cell lacking a chromosome also lacks at least part of the information to produce it again. The experimental criteria of infection or disinfection to prove self-dependence of plasmids have already been discussed, but even if these are satisfied we still cannot assess the degree of autonomy, the relative information inherent in the particle and in the cell, and this remains unknown even for the chromosomes. (There are recurrent hints of cytoplasmic control of genic specificity, Sonneborn, 1954; Michaelis, 1954.) Before we can discuss this question meaningfully, we shall have to learn and adopt a plausible measure of biologically significant complexity. (Cf. the nature-nurture controversy, Hogben, 1951.)

Self-dependence is not only relative to the other-dependence on the cell, but is inherently a statistical concept that should not be used too rigidly. Any configuration of matter is statistically possible: a self-dependent particle may be thought to improve the chances of its own recurrence, but this probability is measured neither 0 without, nor 1 with, the particle. In a complex biological system, the independent emergence of a particle or reaction system might be called a mutation from less differentiated substance, as has happened throughout evolution as well as in controlled experiments, (Spiegelman, 1951; Pollock, 1953).

Self-dependent replication can be attributed to systems that are trivial models of living organisms, because the parts and the product are either both simple or both complex. Crystallization of complex salts and the autocatalytic conversion of trypsinogen to trypsin are examples often quoted; in a universe of IBM machines, a punched card would be a self-reproducing particle, too. If trypsin resynthesized itself from amino acids rather than trypsinogen, we would be more impressed. What is distinctive about organic reproduction, aside from its chemical rather than electromechanical workings, is the gap between the simplicity of the parts and the complexity of the product. Of this, the previously stated criteria for SR tell nothing. Mutability has been suggested as a further criterion, but this is also reducible to a measure of complexity: a particle with only two components that could be independently lost already can manifest four alternative states, one of them null. The full meaning of organic SR, short of its complete description, can perhaps

be developed only in terms of a knowledge of the nutritional input to the particle.

In considering the origin of life, and the possibility of constructing useful models, many authors have postulated the unique, sudden, and improbable creation of a complex living molecule. This *macro*mutational hypothesis is contrary to the contemporary trend of thought which relies upon the concatenation of innumerable, more probable but less ambitious steps to account for further evolutionary development. If the distinction of organic SR is complexity, we might suppose that simple inorganic systems are potential starting points for organic evolution though few have progressed far enough to be recognized as living. The application of binary coding in computing machines illustrates how the most complex information may be expressed as an array of the simplest constituents. In searching for working models of SR, the means by which the *autocatalytic units* can be chemically coordinated to form *SR complexes* may be the most urgent basis of choice of any one among many autocatalytic processes.

BIBLIOGRAPHY

Andrewes, F. W. 1922. Studies in group-agglutination. I. The *Salmonella* group and its antigenic structure. *J. Path. Bact. 28,* 505-521.

Appleyard, R. K. 1953. Segregation of *lambda* lysogenicity during bacterial recombination in *E. coli* K-12. *Cold Spr. Harb. Symp. 18,* 95-97.

Beale, G. H. 1954. *The Genetics of Paramecium Aurelia.* Cambridge Univ. Press, London.

Briggs, R. 1952. An analysis of the inactivation of the frog sperm nucleus by toluidine blue. *J. Gen. Physiol. 35,* 761-780.

Cantino, E. C., and E. A. Horenstein. 1954. Cytoplasmic exchange without gametic copulation in the water mold *Blastocladiella emersonii. Amer. Natur. 88,* 143-154.

Cavalli Sforza, L. L. 1950. La sessualità nei batteri. *Boll. Ist. sieroter. Milan. 29,* 281-289.

Cavalli Sforza, L. L., and R. Ceppellini. 1953. Unicità del Fattore F^+ in *E. coli* K-12. *Notizario di Diagnostica e Terapia. 27,* 3-13.

Cavalli Sforza, L. L., and J. L. Jinks. 1956. Studies on the genetic system of *E. coli* K-12. *Jour. Genet. 54,* 87-112.

Cavalli, L. L., J. Lederberg, and E. M. Lederberg. 1953. An infective factor controlling sex compatibility in *Bacterium coli. J. Gen. Microbiol. 8,* 89-103.

Darlington, C. D. 1944. Heredity, development and infection. *Nature 154,* 164-168.

Demerec, M. 1955. What is a gene?—twenty years later. *Amer. Natur. 89,* 5-20.

Ephrussi, B. 1953. *Nucleo-cytoplasmic relations in micro-organisms.* Oxford Univ. Press, Oxford.

Griffith, F. 1928. The significance of pneumococcal types. *J. Hyg. 27,* 113-159.

Grobstein, C. 1953. Morphogenetic interaction between embryonic mouse tissues separated by a membrane filter. *Nature 172,* 869.

Hayes, W. 1953. The mechanism of genetic recombination in *Escherichia coli. Cold Spr. Harb. Symp. 18,* 75-93.

Hogben, L. 1951. The formal logic of the nature nurture issue. *Acta genetica statistica medica. 2,* 101-140.

Jacob, F., and E. Wollman. 1954. Induction spontanée du développement du bactériophage λ au cours de la recombinaison génétique, chez *Escherichia coli* K-12. *C. R. Acad. Sci. Paris. 239,* 317-319.

King, T. J., and R. Briggs. 1955. Changes in the nuclei of differentiating gastrula cells, as demonstrated by nuclear transplantation. *Proc. Nat. Acad. Sci. U.S. 41,* 321-325.

Lea, D. E. 1947. *Actions of radiations on living cells.* MacMillan, New York.

LeClerc, J. 1954. Etude de la glucose-6-phosphatase dans la membrane chorioallantoïdienne traitée par les microsomes du foie. *Experientia 10,* 251.

Lederberg, E. M. 1951. Lysogenicity in *E. coli* K-12. *Genetics 36,* 560.

Lederberg, E. M. 1952. Allelic relationships and reverse mutations in *Escherichia coli. Genetics 37,* 469-483.

Lederberg, E. M., and J. Lederberg. 1953. Genetic studies of lysogenicity in *Escherichia coli. Genetics 38,* 51-64.

Lederberg, J. 1948. Problems in microbial genetics. *Heredity 2,* 145-198.

Lederberg, J. 1949a. Aberrant heterozygotes in *Escherichia coli. Proc. Nat. Acad. Sci. U.S. 35,* 178-184.

Lederberg, J. 1949b. Bacterial variation. *Ann. Rev. Microbiol.* 1-22.

Lederberg, J. 1951. Genetic studies with bacteria. In *Genetics in the 20th Century,* edited by L. C. Dunn. Macmillan, New York.

Lederberg, J. 1952. Cell genetics and hereditary symbiosis. *Physiol. Rev. 32,* 403-430.

Lederberg, J. 1955. Recombination mechanisms in bacteria. *J. Cell. Comp. Physiol. 45 (Suppl. 2),* 75-107.

Lederberg, J. 1956a. Prospects for a genetics of somatic and tumor cells. *Ann. N.Y. Acad. Sci. 63,* 662-665.

Lederberg, J. 1956b. Conjugal pairing in *Escherichia coli. J. Bact. 71,* 497-498.

Lederberg, J., L. L. Cavalli, and E. M. Lederberg. 1952. Sex compatibility in *Escherichia coli. Genetics 37,* 720-730.

Lederberg, J., and P. R. Edwards. 1953. Serotypic recombination in *Salmonella. Jour. Immunol. 71,* 232-240.

Lederberg, J., and T. Iino. 1956. Phase variation in Salmonella. *Genetics.* In press.

Lederberg, J., E. M. Lederberg, N. D. Zinder, and E. R. Lively. 1951. Recombination analysis of bacterial heredity. *Cold Spr. Harb. Symp. 16,* 413-443.

Lederberg, J., and B. A. D. Stocker. 1955. "Phenotypic" transductions of motility in *Salmonella. Genetics 40,* 581.

Lederberg, J., and E. L. Tatum. 1953. Sex in bacteria: genetic studies, 1945-1952. *Science 118,* 169-175.

Lewis, E. B. 1955. Some aspects of position pseudoallelism. *Amer. Natur. 89,* 73-89.

Link, G. K. K. 1929. Reproduction in thallophytes, with special reference to fungi. *Bot. Gaz. 88,* 1-37.

Luria, S. E. 1947. Recent advances in bacterial genetics. *Bact. Rev. 11,* 1-40.

Luria, S. E. 1953. *General Virology.* Wiley, New York.

Lwoff, A. 1953. Lysogeny. *Bact. Rev. 17,* 269-337.

McClintock, B. 1951. Chromosome organization and genic expression. *Cold Spr. Harb. Symp. 16,* 13-47.

Medawar, P. B. 1947. Cellular inheritance and transformation. *Biol. Rev. 22,* 360-389.

Michaelis, P. 1954. Cytoplasmic inheritance in *Epilobium* and its theoretical significance. *Advances in Genetics 6,* 287-401.

Mitchell, M. B. 1955. Aberrant recombination of pyridoxine mutants of *Neurospora. Proc. Nat. Acad. Sci. U.S., 41,* 215-220.

Morse, M. L. 1954. Transduction of certain loci in *Escherichia coli* K-12. *Genetics 39,* 984-985.

Morse, M. L. 1955. Cis-trans position effect in transduction heterogenotes of *Escherichia coli. Genetics 40,* 586-587.

Morse, M. L., E. M. Lederberg, and J. Lederberg. 1956. Transduction in *Escherichia coli. Genetics. 41,* 142-156.

Muller, H. J., and G. Pontecorvo. 1940. Artificial mixing of incompatible germ plasms in *Drosophila. Science 92,* 418, 476.

Nelson, T. C., and J. Lederberg. 1954. Postzygotic elimination of genetic factors in *Escherichia coli. Proc. Nat. Acad. Sci. U.S. 40,* 415-419.

Pirie, N. W. 1937. The meaninglessness of the terms life and living. In *Perspectives in Biochemistry,* edited by J. Needham and D. E. Green. Cambridge Univ. Press, London.

Pollock, M. R. 1953. Stages in enzyme adaptation. In *Symp. on Bacterial Adaptation.* Soc. for Gen. Microbiol., Cambridge.

Pontecorvo, G. 1952. Genetical analysis of cell organization. *Symp. Soc. Exp. Biol. 6,* 218-229.

Pontecorvo, G. 1954. Mitotic recombination in the genetic systems of filamentous fungi. *Proc. 9th Int. Cong. Genetics.* I. 192-200.

Raper, J. R. 1954. Life cycles, sexuality and sexual mechanisms in the fungi. In *Sex in Microorganisms,* edited by D. H. Weinrich. Pp. 42-81. A.A.A.S., Washington, D.C.

Sonneborn, T. M. 1950. The cytoplasm in heredity. *Heredity 4,* 11-36.

Sonneborn, T. M. 1954. Patterns of nucleocytoplasmic integration in *Paramecium. Proc. 9th Int. Cong. Genetics. I.* 307-325.

Spiegelman, S. 1951. The particulate transmission of enzyme-forming capacity in yeast. *Cold Spr. Harb. Symp. 16,* 87-98.

Spratt, N. T., Jr. 1954. Physiological mechanisms in development. *Physiol. Rev. 34,* 1-24.

Stent, G. S., and E. L. Wollman. 1950. Studies on activation of T_4 bacteriophage by cofactor. II. The mechanism of activation. *Bioch. Bioph. Acta 6,* 307-316.

Stocker, B. A. D. 1949. Measurements of rate of mutation of flagellar antigenic phase in *Salmonella typhi-murium. J. Hyg. 47,* 398-413.

Stocker, B. A. D., N. D. Zinder, and J. Lederberg. 1953. Transduction of flagellar characters in *Salmonella. J. Gen. Microbiol. 9,* 410-433.

Sturtevant, A. H., and J. Schultz. 1931. The inadequacy of the sub-gene hypothesis of the nature of the scute allelomorphs of *Drosophila. Proc. Nat. Acad. Sci. U.S. 17,* 265-270.

Sussman, M., and R. Sussman. 1956. Cellular interactions during the development of the cellular slime molds. This symposium. Pp. 125-154.

Tatum, E. L., and J. Lederberg. 1947. Gene recombination in the bacterium *Escherichia coli. J. Bact. 53,* 673-684.

Wagner, R. P., and H. K. Mitchell. 1955. *Genetics and metabolism.* Wiley, New York.

Weiss, P. 1947. The problem of specificity in growth and development. *Yale J. Biol. Med. 19,* 235-278.

Wheeler, H. E. 1954. Genetics and evolution of heterothallism in *Glomerella. Phytopathology 44,* 342-345.

Wiedling, S. 1948. Beitrage zur Kenntnis der vegetativen Vermehrung der Diatomeen. *Botaniska Notiser,* pp. 322-354.

Wilson, E. B. 1931. The distribution of sperm-forming materials in scorpions. *J. Morph. 52,* 429-483.

Wollman, E. 1953. Sur le déterminisme génétique de la lysogénie. *Ann. Inst. Pasteur. 84,* 281-293.

Wollman, E., and F. Jacob. 1955. Sur le mécanisme du transfert de matériel génétique au cours de la récombinaison chez *Escherichia coli* K-12. *C. R. Acad. Sci. Paris. 240,* 2449.

Zinder, N. D., and J. Lederberg. 1952. Genetic exchange in *Salmonella. J. Bact. 64,* 679-699.